Effects of Stenting the Parent Artery on Aneurysm Filling and Gene Expression of Various Potential Factors Involved in Healing of Experimental Aneurysms

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Summary

Intracranial stents are increasingly used in the endovascular treatment of aneurysms, but very little is known regarding their effect on the cellular and molecular evolution of aneurysms.

Bilateral venous pouch lateral wall carotid aneurysms were created in 20 dogs. All dogs then underwent angiography and balloon-expandable stenting of one aneurysm four to six weeks later. Fifteen dogs underwent aneurysm harvesting at one day (n=3), four days (n=4), seven days (n=3), and 14 days (n=5) for mRNA expression analysis, using axial sections taken from the aneurysm neck and fundus for RT-PCR amplification of four cytokines or growth factors: TNF-a, TGF-b$_1$, MCP-1, and PDGF-BB; two adhesion molecules: VCAM-1 and PECAM-1; five matrix modifying agents; MMP-2, 9, TIMPs 1, 3, 4, and two cellular markers: CD34 and a-SMA.

Five other dogs, sacrificed at 12 weeks, were examined for extent of filling of the aneurysm neck with organized tissue and for neointima formation at the aneurysm ostium. Angiography was performed prior to sacrifice in all animals, and compared with initial studies. Eleven out of 20 stented aneurysms showed a favorable angiographic evolution, while none of the 20 non-stented aneurysms improved (p=0.001). Pathology showed partially occluded aneurysms, with neointima formation around the stent struts. Observed trends in mRNA expression, that stenting increased expression of genes involved in organization and neointima formation, agreed with experimental hypotheses, but differences between stented and non-stented aneurysms did not reach statistical significance.

Parent vessel stenting was associated with angiographic improvement of aneurysm appearance. Modifications in mRNA expression patterns following stenting deserve further study to better establish potential molecular targets to promote aneurysm healing.

Introduction

Endovascular coiling of intracranial aneurysms is increasing in popularity following the publication of the results of the ISAT[1], but this technique is too often associated with recurrences[2]. Attempts to decrease recurrences have triggered the development of several technical and bioengineering innovations[3-7], including endoluminal stenting.

Currently, stents are most often used as an adjunct to coiling, but stents by themselves have been reported to lead to aneurysm occlusion[8-10]. Beyond preventing coil herniation, the beneficial effects of stents are likely realized by modifying flow; they modify intra-aneurysmal hemodynamics, may provoke aneurysm throm-

bosis, re-establish normal blood flow patterns within the parent vessel, and perhaps buffer the hemodynamic forces implicated in recurrences [11-15]. In addition, the stent may provide a supportive scaffold for neointima deposition at the junction of the parent vessel and aneurysm neck.

The feasibility of treating experimental aneurysms with stents has been established using angiographic and pathologic studies [16,17], but molecular clues accounting for the variable treatment outcomes remain elusive. Conceptual and molecular parallels likely exist between i) vascular wound healing, organization and neointima formation within the body and neck of the aneurysm [18]; ii) angiogenesis and recanalization at the aneurysm neck [19-21]; and iii) molecular effects of flow modification previously associated with atheroma or stenting [13,14].

For neointima and organization to completely fill the defect formed by an aneurysm, the provisional matrix formed by fibrin and platelets must be invaded and replaced by neointimal cells or myofibroblasts and secreted extracellular matrix [18]. In balloon-injury models, the process resulting in neointima formation relies on complex signaling cascades and cellular proliferation, establishment of cell-cell and cell-matrix connections, and matrix degradation and reformation [22]. At the aneurysm neck, another condition for stable occlusion is the absence of recanalizing channels within the thrombus [19-21]. By altering flow with a stent, we aim to observe changes in genetic expression of key factors potentially involved in neointima formation/-thrombus organization or recanalization.

The genes we chose to follow can be classified into growth factors and cytokines (PDGF-BB, TGF-β_1, TNF-α, MCP-1), adhesion molecules (integrin $\alpha v \beta_{III}$, VCAM-1, PECAM-1), matrix metalloproteinases and their inhibitors (MMP-2 and 9, and TIMP 1, 3, and 4), and the cellular marker α-SMA. To study the process of formation of recanalizing channels, we focused on the expression CD34, as well as MCP-1 and the MMPs / TIMPs.

Determination of gene expression changes that are associated with favourable angiographic and pathological results promises to usher in a new era of molecular-based aneurysm treatment, and stenting offers a unique opportunity to study the mechano-sensitive changes in mRNA expression occurring within an aneurysm following endovascular therapy.

Material and Methods

Protocols were approved by the Institutional Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care. All procedures were performed under general anaesthesia. Twenty beagles weighing 8-12 kg were sedated with acepromazine (0.1 mg/kg), glycopyrrolate (0.01 mg/kg), and butorphanol (0.1 mg/kg), and anaesthesetized with intravenous thiopental (15 mg/kg). Animals were ventilated artificially and maintained under anaesthesia with 2% isoflurane. Post-operative analgesia was provided for three days with a 50 µg Fentanyl patch.

We used the lateral wall carotid aneurysm model of German and Black, as modified by Graves [20,23]. Briefly, the carotid artery was accessed through a midline incision. A portion of the left external jugular vein was harvested and divided in half to form venous pouches. Temporary aneurysm clips were applied to the proximal and distal carotid, and a 6 mm arteriotomy was created. The vein pouch was attached to the artery via an end-to-side anastomosis with running 7.0 Prolene suture, the apex of the pouches sealed with hemostatic clips, and circulation restored.

After a 4-6 week period to permit post-surgical changes to abate, the endovascular procedure was performed. Following transfemoral angiography, a coronary balloon-expandable stent ranging in length from 13-23 mm and in diameter from 3.0-4.5 mm was positioned to bridge one aneurysm neck and deployed. The contralateral side subsequently underwent balloon angioplasty using the same balloon used to expand the stent. Animals did not receive any antiplatelet agents or anticoagulants. Angiography was repeated immediately following stenting and prior to sacrifice. Fifteen animals underwent a separate harvesting procedure at one day (n=3), four days (n=4), seven days (n=3) and 14 days (n=5) post-stenting, where the portions of the carotid arteries bearing the aneurysms were removed under anaesthesia. Animals were sacrificed by barbiturate overdose. An additional five dogs were sacrificed at 12 weeks for pathological analysis; their aneurysms were removed and fixed in formalin.

The evolution of aneurysms following treatment was evaluated by examining the angiographic progression between initial and final angiograms. A binary score of better versus

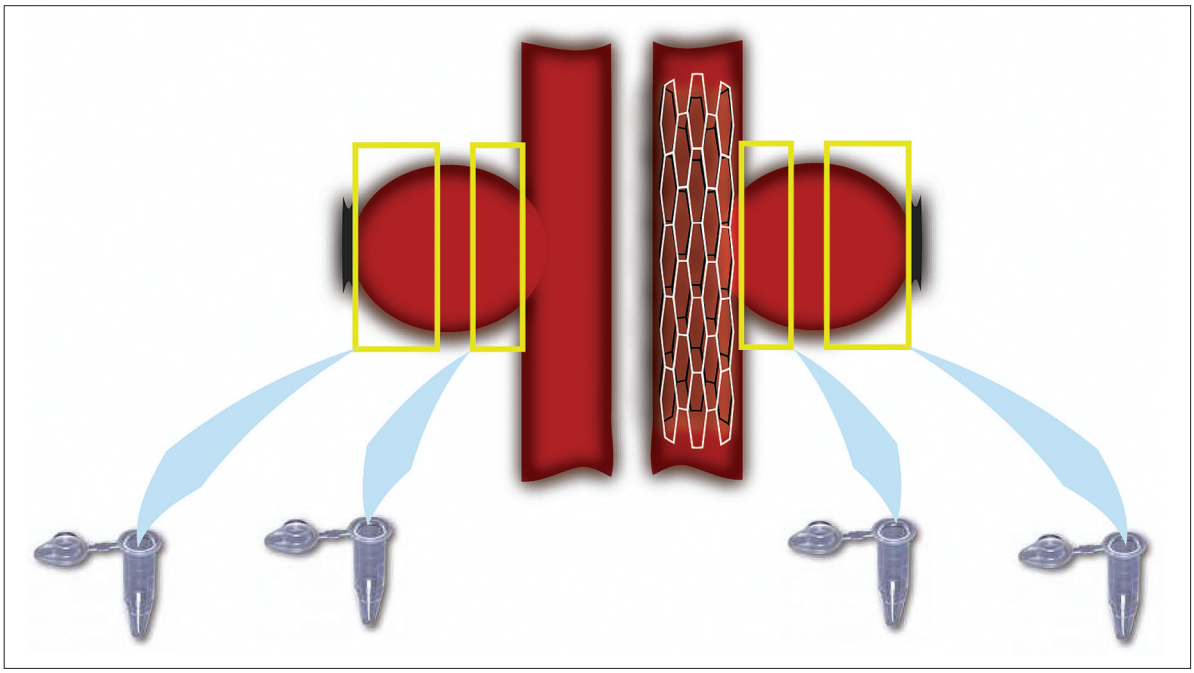


Figure 1 Schematic of tissue chosen for mRNA expression studies: 2 and 3 mm axial sections of aneurysm tissue, from the neck and fundus, respectively, were harvested.

same/worse was given, based on whether the final aneurysm opacified less, equal to or greater than on the initial angiogram following stenting.

Pathological specimens were prepared by cutting the stent/artery complex longitudinally, followed by "en face" photography of the aneurysm ostium (Clemex, Quebec, Canada). Pathological findings were studied after formalin fixation and hematoxylin-phloxine-saffron (HPS), Movat's pentachrome, and immunohistochemical staining.

The fifteen animals analyzed for mRNA expression had each aneurysm cut at the junction with the parent carotid, taking care not to include carotid tissue. Two mm and three mm axial sections of the aneurysm neck and fundus, respectively, were harvested (figure 1). The tissue samples included the vein pouch aneurysm wall as well as any formed thrombus or tissue adherent to the wall. Samples were frozen with liquid nitrogen. Isolation of total cellular RNA was performed using the phenol method. Aliquots of 1 to 3 µg were used for first strand cDNA synthesis using Superscript II reverse transcriptase (Invitrogen, Canada) and PCR amplifications were performed with Platinum Taq polymerase (Invitrogen). After determining the linear range for each target gene, amplification of the gene under study was carried out using the primer sequences listed in table 1. Thermal cycling conditions consisted of enzyme activation at 94°C for one minute, then a cycle of denaturation also at 94°C for one minute, annealing as described in table 1 and extension at 72°C, each for one minute, repeated 40 times and 35 times for GAPDH. This was followed by a final extension at 72°C for one minute. PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide and UV transillumination. Quantitative analysis was carried out using a computerized densitometric imager (ImageQuant; Amersham Biosciences, Canada) to obtain gene/GAPDH ratios and thus correct for the variable amounts of tissue within the specimens. For each gene, the mean mRNA expression of the 14 genes at the four different time points was quantified for the neck and fundus of each stented aneurysm, and compared to that from the corresponding control aneurysm.

Statistics

Angiographic results were divided into two groups: a) decreased opacification b) unchanged or increased size. These groups were com-

Table 1 **Sequences of primers of selected genes chosen for RT-PCR.**

	Gene	Tm	NT(bp)	Foward primer	Reverse primer	Type
markers	CD-31	52	687	5'-CCCAGGGTGACACTGGACAA-3'	5'-CCTTCTGGATGGTGAAGTTGG-3'	Consensus human/mice
	CD-34	64	434	5'-GCCCAGTCTGAGGTGAGGCCTCA-3'	5'-CAGGTGTTGTCTTGCTGAATGG-3'	Dog (NM 001003341.1)
	α_v	64	622	5'-GTCAAGGAGGATTCAGCATTGAT-3'	5'-AGCAGCAATTGCAATATCATTGA-3'	Consensus human/mice
	VCAM	58	694	5'-CTCTTGGAGAACCCAGATAGACAGTC-3'	5'-ATGTTCCAGAATCTTCCAGCCTCATAGCAATTA-3'	Dog (NM 001003298.1)
Reporter	GAPDH	64	500	5'-GCCAAAAGGGTCATCATCTC-3'	5'-GCCCATCCACAGTCTTCT-3'	Consensus human/mice
MMPs	MMP-2	58	580	5'-ATGCAGAAGTTCTTTGGGCTGC-3'	5'-TTGCCATCCTTCTCAAAGTTGTA-3'	Dog (AF 177217)
	MMP-9	64	476	5'-TATGACACCGACCGTCGGTTC-3'	5'-GTACATGAGCGCTTCTGGCAC-3'	Dog (AF 169244)
TIMPs	TIMP-1	52	341	5'-GCGTTATGAGATCAAGATGAC-3'	5'-CTGGTCCGTCCACAAGCA-3'	Dog (NM 001003182.1)
	TIMP-3	52	260	5'-TACCAGTACCTGCTGACAGG-3'	5'-GCCCATCCTCGGTACCAGCT-3'	Dog (XM 53840.2)
	TIMP-4	58	331	5'-ATCTCCAGTGAGAAGGTAGT-3'	5'-GTGGTGATTTGGCAGCCACACAGTTC-3'	Consensus human/mice
Growth	TGFβ1	64	366	5'-TTCCTGCTCCTCATGGCCAC-3'	5'-GCAGGAGCGCACGATCATGT-3'	Dog (NM 001003309)
Factor	PDGFbb	64	393	5'-ATGAATCGCTGCTGGGCGCTCTTCCT-3'	5'-GGAGCAGCGCTGCACCTCCAC-3'	Dog (NM-001003383)
	TNFα	64	436	5'-CCTCCAACTAATCAGCCCTCT-3'	5'-TCTCAGCGCTGAGTCGATCA-3'	Dog (NM 001003244.1)
	MCP1	64	305	5'-ATGAAGGTCTCCGCAGCGCTC-3'	5'-TCATGGCTTTGCAGTTTGGGTTTG-3'	Consensus human/mice

pared with the McNemar two-tailed test. mRNA expression results were treated with ANOVA repeated measurements for three factors: stent, time, and location (neck or fundus), using the Procedure Mixed program of SAS software, version 9. $P < 0.05$ was considered to be significant for all tests.

Results

A) Surgical and Endovascular Results

All animals tolerated the surgical and endovascular procedures well. Stent placement was deemed adequate in all cases, and resulted in an immediate increase in blood transit time through the aneurysm, as qualitatively assessed by the observer.

1. Angiographic results:

Of 40 aneurysms created in 20 dogs, 37 were patent at initial angiography at four to six weeks, for a spontaneous occlusion rate of 7.5%. Stent deployment had an effect on angiographic progression (figure 2); 11 of 20 stented aneurysms were angiographically better, while none of the control aneurysms improved (p=0.001). Only one of the stented aneurysms became completely obliterated on angiography at 12 weeks.

2. Pathology results:

Macroscopically, stenting seemed to redefine the endoluminal boundary, with neointima formation up to and around the stent struts at least in some cases (11/20) (figure 3). In other cases (9/20), stented aneurysms remained widely patent. Intermediate cases of incompletely occluded aneurysms showed small channels connecting the parent vessel with dilated openings within the organizing intra-aneurysmal thrombus. Non-stented control aneurysms were usually widely patent, while some had partial filling of the fundus with organized tissue.

B) mRNA Expression Results

RT-PCR results in stented and control aneurysms are illustrated in figure 4 and summarized in table 2.

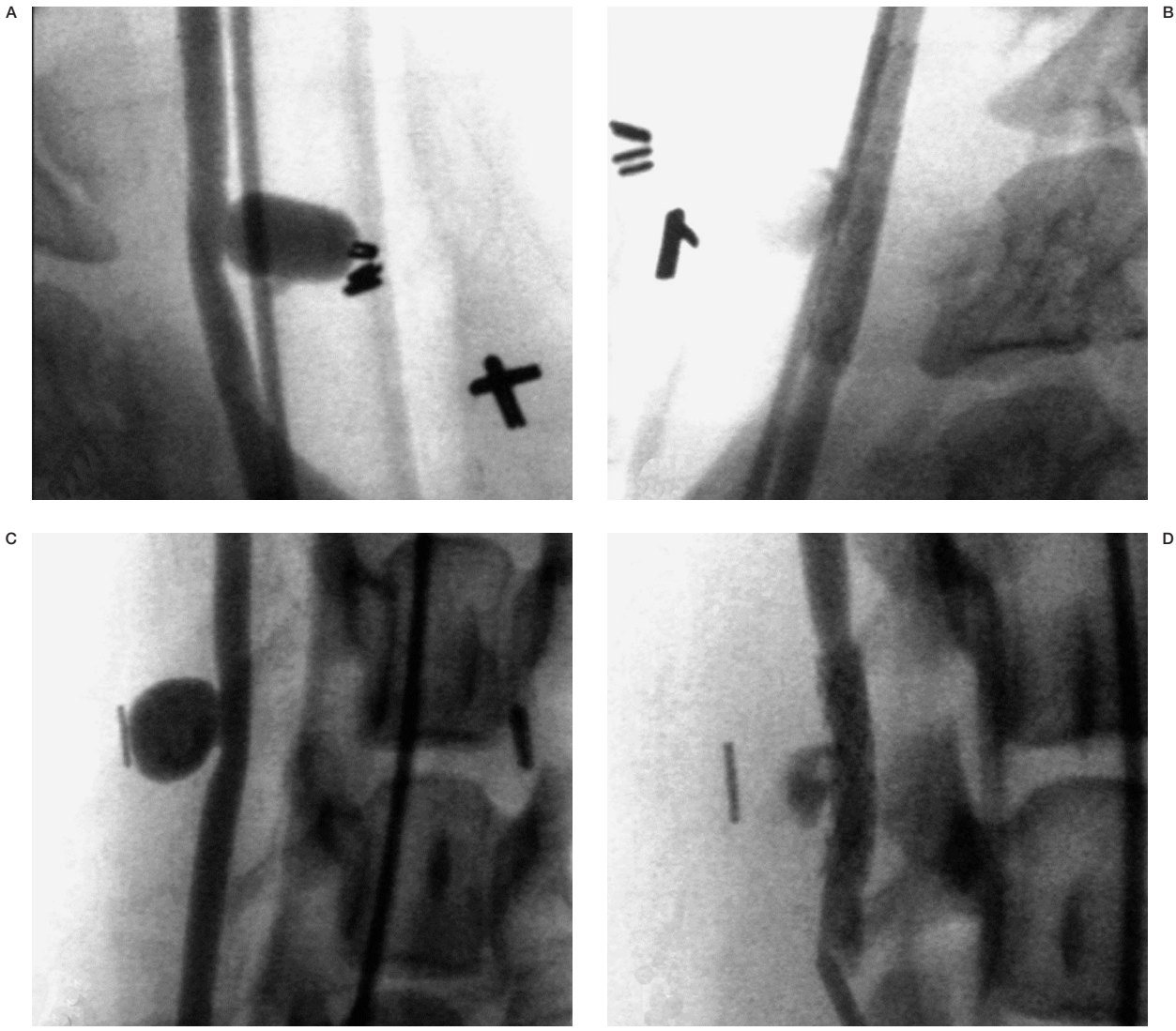


Figure 2 Angiographic progression of stented lateral wall aneurysms with time. A,C) represent initial images at 4-6 weeks following surgical creation. B,D) show improved angiographic appearance following stenting, but not complete obliteration, at 4 days and 12 weeks respectively.

1. Growth factors and cytokines

The kinetic profile of TGF-β_1 expression at the aneurysm neck showed an initial decrease, reaching a nadir at four days, with a subsequent slow continuous increase in expression to 14 days, with only slightly greater expression for stented aneurysms compared to non-stented aneurysms. TGF-β_1 expression in the tissue from the fundus was remarkably similar between stented and non-stented aneurysms. Both showed a decrease similar to that of the tissue from the neck, with a more delayed nadir at seven days.

PDGF-BB expression of neck tissue for stented aneurysms remained fairly constant for seven days, followed by an increase at 14 days. During this interval, non-stented neck expression of PDGF-BB decreased until the seven day mark, and then increased to 14 days. At the level of the aneurysm fundus, stented aneurysms showed a small decrease until seven days, with subsequent increase. Non-stented aneurysms showed a small increase, peaking at four days and then tapering off.

The peak expression at of TNF-α at four days is compatible with inflammatory cell infil-

tration and secretion. We observed almost parallel TNF-α expression curves for both stented and non-stented controls at the neck and fundus. Stented fundus showed a slightly higher peak at four days and a deeper nadir at seven days than controls.

MCP-1 expression at the neck was lower for stented aneurysms at all time points compared to controls. At the fundus, the stent led to decreased MCP-1 expression compared to controls over the first seven days, which slowly increased to become greater than controls at 14 days.

The differences in mRNA expression between stented and non-stented aneurysms were not statistically significant for any growth factors or cytokines assayed at either the neck or the fundus.

2. Adhesion molecules

PECAM-1 expression was variable at the different time-points, showing a biphasic increasing pattern for neck tissue stented aneurysms, with a small peak at four days, while non-stented tissue at the neck and stented and non-stented fundus tissue all showed sharply decreasing expression initially that subsequently tapered off at 14 days.

Table 2 **Genes potentially involved in the molecular mechanisms of aneurysm healing, with the hypothesized expression changes obtained from a survey of the literature of other vascular processes, and our experimental observations.**

	Expression Changes Due to Stent	
GENE	**Hypothesized**	**Observed**
TGF-b	↑	↑
PDGF-BB	↑	↑
MCP-1	↑	↓
TNF-a	↑	↓
VCAM-1	↑	-
MMP-2	↑	↑
MMP-9	↑	↑
TIMP-1	↓	↓
TIMP-3	↓	↓
TIMP-4	↓	↓

We observed only small increases in VCAM-1 expression for stented aneurysms at the neck and fundus over the first seven days, which subsequently normalized. Non-stented neck and fundus tissue expressed more VCAM-1 than stented tissue over the first seven days; this trend was reversed for days seven to 14.

We found that integrin α_{VI} expression of neck tissue steadily increased from one day to seven days, followed by a return to low levels of expression at 14 days. During this interval, the expression of integrin α_{VI} of non-stented control necks increased rapidly, peaking at four days, and then rapidly dropped to baseline by seven days, remaining at low levels. For both stented and non-stented fundus tissue, a very mild increase in expression occurred over the first seven days, followed by a slow return to baseline by 14 days. None of the different changes in mRNA expression between stented and non-stented controls were statistically significant for any adhesion molecules.

3. MMPs / TIMPs

MMP-2 expression at the neck of stented aneurysms increased to a peak at four days and remained elevated, gently decreasing to 14 days. Non-stented neck tissue showed a steady, continuous decrease in MMP-2 expression from day one to day 14. The curves for MMP-2 expression at the fundus were very similar between stented and non-stented groups, with a slight predominance in stented MMP-2 expression beginning on day four and continuing until day 14.

MMP-9 expression of stented neck tissue increased sharply at four days and quickly returned to baseline. Non-stented control neck tissue, as well as fundus tissue from stented and non-stented aneurysms remained at a low level of MMP-9 expression across all time points.

TIMP-1 expression curves were almost parallel for stented and non-stented groups at the neck and fundus. Expression decreased rapidly over the first seven days, followed by a recovery at 14 days. TIMP-1 expression was slightly less for stented necks compared to all other groups.

TIMP-3 expression increased for all groups to a small peak at four days, followed by a decrease to baseline by seven days, remaining at this low level until 14 days. TIMP-3 expression was slightly less for stented necks compared to all other groups.

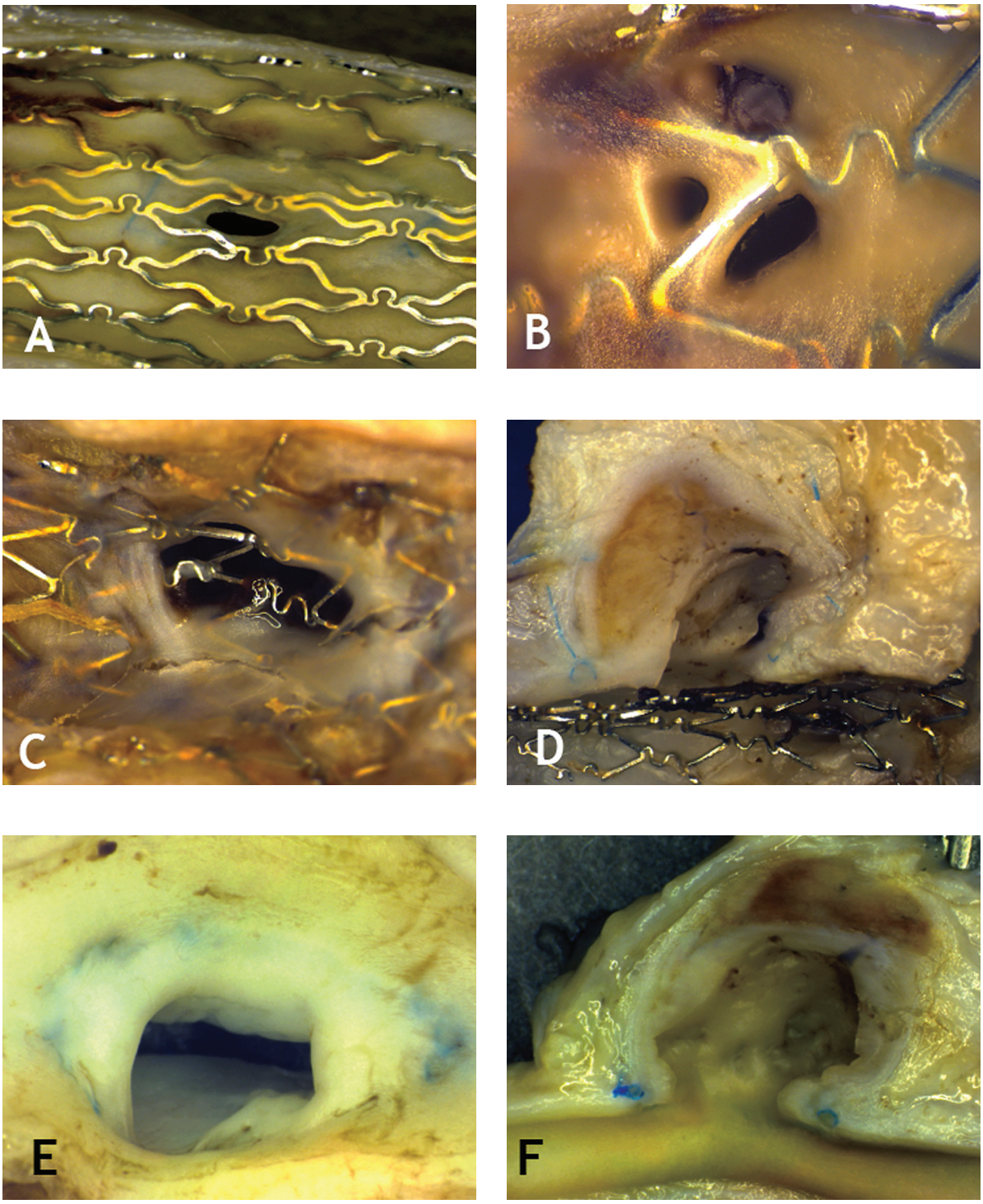
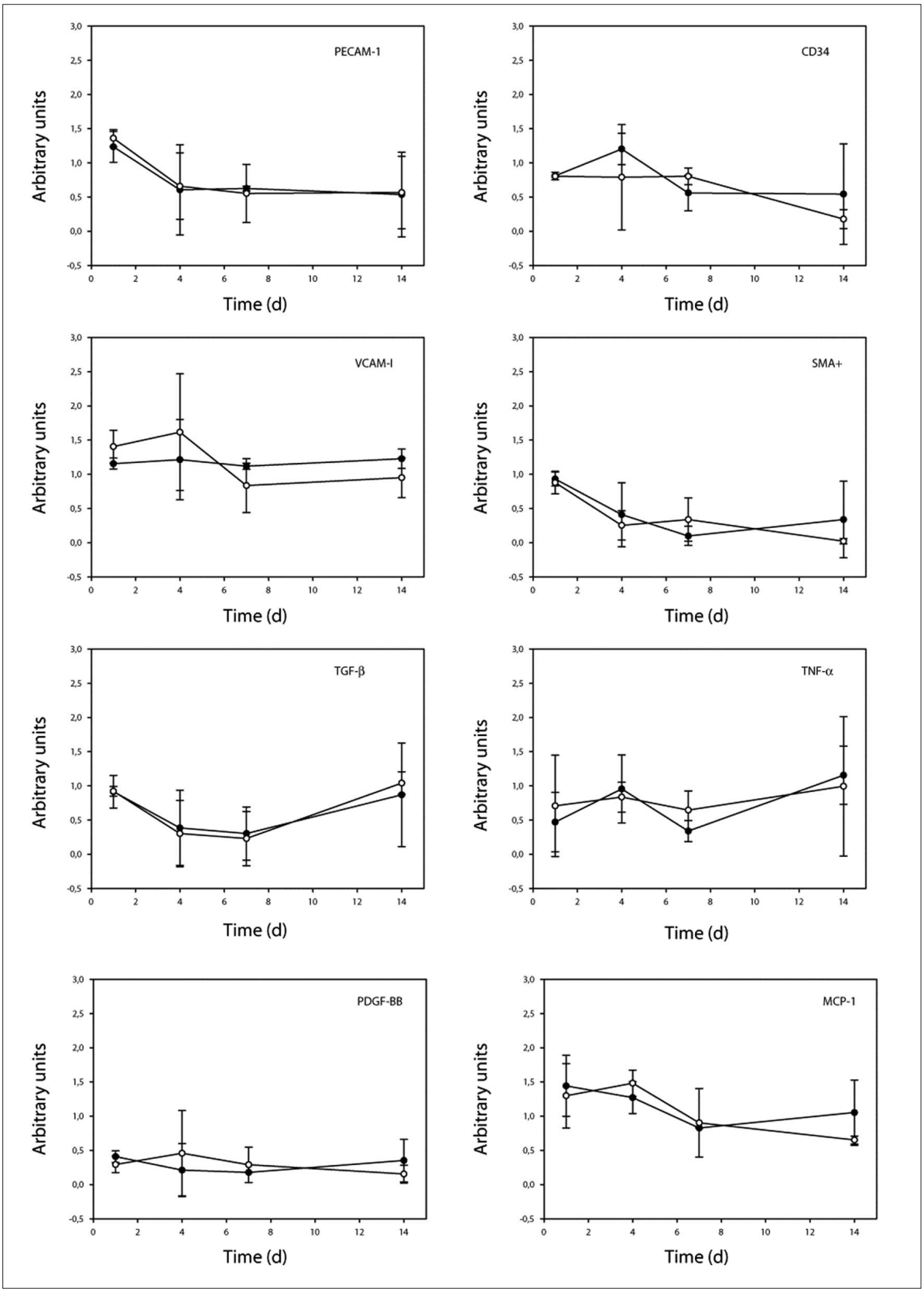


Figure 3 Photographs of stented (A-D) and non-stented (E-F) control aneurysms. Good neointima formation in (A, B), filling aneurysm body and neck and incorporating stent struts. C, D) reveal incomplete neointimal filling, with the aneurismal defect evident on en-face as well as sagittal views. E, F) demonstration the widely patent ostia and minimal neointimal filling typical of non-stented control aneurysms.

A


PECAM-1
CD34
VCAM-I
SMA+
TGF-β
TNF-α
PDGF-BB
MCP-1
Arbitrary units
Time (d)

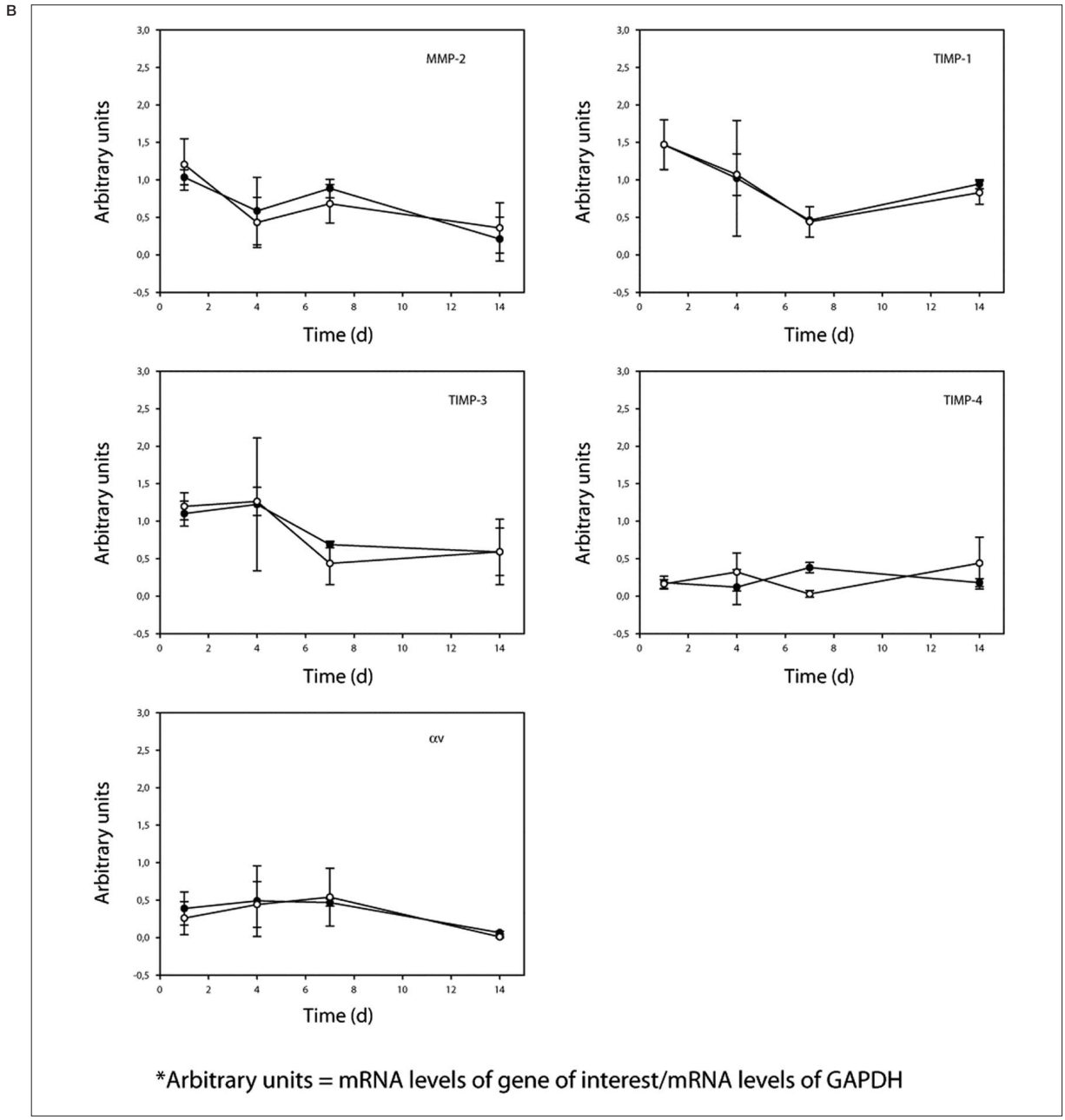


Figure 4 Gene expression associated with stenting. Results of RT-PCR analyses, expressed as specimen/arterial controls ratio, according to time. A) Gene expression at the fundus B) Gene expression at the neck of aneurysms. = Stented = Not stented).

TIMP-4 expression profiles were almost parallel for both stented and non-stented necks, with expression levels slightly lower for stented necks.

At the fundus, TIMP-4 expression levels of stented and non-stented aneurysms were mirror images of each other; stented tissue decreased at four days, followed by a peak at seven days with a return to baseline by 14 days, while non-stented fundus tissue peaked at four days, dropped at seven days, and then became elevated again at 14 days.

The differences in mRNA expression between stented and non-stented controls were not statistically significant for any of the MMPs or TIMPs.

4. Cellular markers

Neck tissue from stented aneurysms showed a steady increase in α-SMA expression over the first four days, which then leveled off. α-SMA expression of non-stented necks was lower than for stented necks for all time points except at one day, a time-point for which we had difficulty with mRNA degradation and sample loss. Tissue at the fundus also showed an overall decrease in α-SMA expression for stented and non-stented controls without clear differences in expression profiles.

Both stented and non-stented aneurysm necks showed increasing CD34 expression, reaching the same peak at four days, with subsequent slow return to baseline over 14 days. Non-stented fundus tissue did not show the same peak at four days, remaining at a level of expression closer to baseline.

The difference in α-SMA or CD34 mRNA expression between stented and non-stented aneurysms was not statistically significant for tissue from either the aneurysm neck or fundus.

Discussion

The salient findings from this work are that stents improve the angiographic and pathologic appearance of lateral wall aneurysms in 55% of cases, and that this improvement correlated with several mRNA expression trends of genes implicated in neointima formation and thrombus organization: i) increased expression of the growth factors TGF-β_1 and PDGF-BB for aneurysm neck tissue starting at four days; ii) decreased expression of the pro-inflammatory and chemotactic agents TNF-α and MCP-1, and iii) increased expression of MMP-2 and MMP-9 at the aneurysm fundus, as well as decreased TIMP-1, 3, and 4 at the aneurysm neck. The improved neointima formation was further reflected in a trend towards increased α-SMA expression of tissue from the neck of stented aneurysms. There were no observed trends in mRNA expression of PECAM-1, VCAM-1, or integrin α_v to suggest a role for adhesion molecules to account for the improved appearance of stented aneurysms.

The contribution of stenting to aneurysm healing is difficult to assess when stents are used as adjuncts to coiling. The observed angiographic improvement following stenting suggests that their role in aneurysm healing may extend beyond prevention of coil herniation; we hypothesized that this effect was at least in part mediated by flow disruption with subsequent changes in genetic expression. Hypothetical stent-related modifications of gene expression are summarized in table 2.

TGF-β_1 is a multifunctional regulator; its actions are dependent on species, cell phenotype, growth conditions, and interaction with other growth factors [24,25]. In atherosclerosis, with disrupted blood flow patterns, TGF-β_1 promotes lesion formation and vascular smooth muscle cell (VSMC) proliferation [26,27]. Alterations in shear stress can upregulate TGF-β_1 expression [28], and we were able to detect a non-significant increase in TGF-β_1 expression at the aneurysm neck following stenting, which may provide a molecular explanation for the increased neointima formation observed at pathology.

PDGF-BB is a potent chemoattractant and mitogen produced by activated platelets and macrophages that stimulates VSMC proliferation and migration [22]. Experiments that surgically decreased blood flow in an animal model increased PDGF-BB expression [29]. Cultured endothelial cells increased PDGF-BB expression in response to increased shear stress and turbulent blood flow [30]. The marginal increase in PDGF-BB expression in stented aneurysm neck and fundus tissue beginning after seven days may have contributed to increased neointimal formation.

The pro-inflammatory effects of TNF-α are well-characterized [31-33]. This cytokine is produced by macrophages, neutrophils, endothelial cells and VSMCs, and exerts multiple biological effects on a multitude of cell types [31,33]. TNF-α locally augments the expression of adhesion molecules and MCP-1 and attracts leukocytes. TNF-α also modulates growth factor expression, stimulates MMP activity, and can trigger endothelial cell apoptosis [33,34]. TNF-α has been implicated in the generation of atherosclerotic lesions at the site of turbulent blood flow; laminar blood flow inhibits TNF-α expression [32], whereas atheromatous areas characterized by turbulent blood flow have high TNF-α levels [35]. Because stents disturb blood flow, we had expected stenting to increase TNF-α expression, and for this to increase expression of adhesion molecules. We did not however observe expression changes of TNF-α or adhesion molecules.

MCP-1 is implicated in the formation of atherosclerotic lesions [36], neointimal hyperplasia following balloon injury [37,38], and may also play

a role in the recanalization of organized intravascular thrombi[21]. Laminar flow inhibits MCP-1 expression[39], and we had anticipated that flow disruption due to stenting would increase MCP-1 expression. As with TNF-α, we observed a trend towards lower expression levels of MCP-1 for stented aneurysms at all time points compared to controls. Changes in MCP-1 expression are known to occur ultra-early (within five hours) in cultured endothelial cells[40], and so it is possible that the time-points we chose may have missed important early events. Nonetheless, because MCP-1 is also implicated in promoting the recanalization of organized thrombus (and thus potentially, recurrences), a relative decrease in expression of this chemokine may prove beneficial.

The balance of MMP and TIMP activity is essential to maintain the proper 'fluidity' of the extracellular matrix[41]. The activity of the collagenases MMP-2 and MMP-9 is thought to be essential to permit cellular migration[25] of neointimal cells into the provisional matrix of platelets and fibrin within a treated aneurysm. MMPs are also essential to the process of angiogenesis[42], and contribute to the formation of neovessels within organizing thrombi. A strategy to decrease the incidence of aneurysm recurrences due to the formation of recanalizing channels or neovessels connected to the parent vessel could aim to inhibit MMP or promote TIMP activity. Proper timing of intervention would be necessary to ensure that sufficient neointimal cells had migrated into the provisional matrix prior to form a robust neointima prior to altering the balance of MMP/TIMP activity to minimize the formation of recanalizing neovessels.

MMPs can also modulate signal transduction pathways by acting on inflammatory mediators, growth factors, and growth factor receptors[43]. MMP-2 and MMP-9 production and activation are increased following arterial balloon injury and subsequent remodeling[44,45], and MMP-9 has been localized to atherosclerotic plaques in humans[46]. In an in vitro model, elevated shear stress induces changes in MMP-2 and MMP-9 expression that follow different temporal patterns: two days after shear onset, MMP-2 expression levels increase and remain elevated, while MMP-9 peaks quickly and decreases[25]. MMP-9 expression is also responsive to shear pattern, with oscillatory or turbulent blood flow resulting in greater, more sustained expression compared to unidirectional shear[47]. We anticipated that the flow disturbances caused by stenting would increase expression of both MMPs, with a more sustained elevation for MMP-2 than MMP-9. We further anticipated a decrease in TIMP activity to result in a net decrease in the MMP/TIMP balance.

We observed only small increases in MMP-2 activity in the necks and fundi of stented aneurysm compared to controls, which started after four days. Because MMPs are potent enzymes, perhaps only small changes in MMP expression, in conjunction with the observed decreased TIMP expression may be sufficient to lead to matrix degradation and allow greater neointimal cell migration and promote aneurysm healing. The sharp, transient increase in MMP-9 expression at four days for stented neck tissue was in agreement with published findings, and may reflect the increased exposure of cells of the aneurysm neck to the mechanical force of blood flow.

The α-SMA cells were anticipated to follow the pattern of migration observed in arterial balloon injury models, where cells begin to proliferate in the media after approximately 24 hours[48], then migrate into the intima at four days, continue to proliferate and form extracellular matrix for at least three months[49]. In our experiment, we hypothesized that the stent would provoke thrombosis within the aneurysm, and the expression of α-SMA would increase as the cells that contribute to neointima formation populate the provisional matrix formed within the thrombosed aneurysm. With the exception of the time-point at one day, the kinetic profile of α-SMA expression for stented tissue at the neck was in accordance with expectations for progressive thrombus invasion with cells destined to populate and from the neointima. The presence of these cells is likely of particular importance at the neck of endovascularly treated aneurysms, to promote robust neointima formation at the junction of the parent vessel and aneurysm neck.

As observed with the resolution of deep venous thrombi, the process of recanalization within and around the formed intra-aneurysmal thrombus may contribute to aneurysm recurrences. To investigate the molecular aspects of this process, we followed expression levels of MCP-1, MMPs/TIMPs, and CD34. The chemokine MCP-1 has recently been implicated to stimulate the monocytic drilling of 'tunnels'

within thrombi [50], and the uniformly lower expression of MCP-1 of stented aneurysm neck tissue may partially account for the better angiographic and pathologic outcomes observed at 12 weeks.

There was no reliable means to differentiate MMP or TIMP activity needed for neointimal cell migration from that of neovessel formation. By observing the kinetics of TIMP expression following aneurysm treatment, the initial decrease in expression lasted seven days before increasing again. Perhaps this period of time is sufficient to allow for neointimal cell migration, and future pharmacologic interventions to alter the MMP/TIMP balance to prevent recurrences might be timed for seven days post-stenting.

CD34 expression was assayed to search for a possible increase in cells of endothelial lineage within the aneurysmal thrombus, particularly at the neck. Neovessels propagating through the thrombus could originate from the tissues surrounding the aneurysm aiming to provide blood supply to the neointimal cells; alternatively, they could represent nefarious recanalizing channels originating from the parent vessel. Circulating endothelial progenitor cells may also contribute to the observed CD34 expression. The CD34 expression profiles of stented and non-stented aneurysms did not clarify the role of recanalization in aneurysm recurrences in this experimental model.

The greatest limitation of this experiment was the small number of dogs we used. As with all animal experimentation, we are limited by ethical and practical considerations; given the size of the effects we observed here, perhaps 50 or more animals would have been needed to attain statistical significance, a sacrifice we could not justify given the lack of indications of where potentially findings were. Other sources of variation of our model include the variability of positioning of the stent struts relative to the aneurysm ostium, which may have resulted in inconsistent modulation of the fluid dynamics and relatively large changes in mRNA expression. Although we corrected for cellularity by using GAPDH:gene ratios, variability in the formation of thrombus within the aneurysmal pouch may have affected the mRNA expression results. More refined molecular tools also remain to be developed for use in the canine model. Alternatively, we may have omitted some key molecular factors that change dramatically in response to mechanical forces, or our window of sampling may have missed important ultra-early or late events.

The cellular composition of a venous pouch aneurysm is different from that of a naturally occurring aneurysm, and the local environment of the experimental aneurysms certainly differed from subarachnoid aneurysms. Finally, balloon-expendable coronary stents are not alike currently available self-expendable intracranial stents.

Conclusions

Parent vessel stenting is insufficient to cause complete occlusion of lateral wall aneurysms when used alone. However, stenting does contribute to the healing process, as evidenced by the improvement in angiographic and pathologic appearance in 55% of cases. We were unable to detect statistically significant stent-related differences in mRNA expression, although we observed trends in expression of growth factors, cytokines, and matrix agents that may prove useful to the future design of a molecular-based therapy for aneurysms.

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